

promoter, a reverse transcriptase, a DNA polymerase, an RNA degrading enzyme, NTPs and dNTPs, in addition to the detection components outlined below.

[0175] These components result in a single starting RNA template generating a single DNA duplex; however, since this DNA duplex results in the creation of multiple RNA strands, which can then be used to initiate the reaction again, amplification proceeds rapidly.

[0176] As outlined herein, the detection of the newly synthesized strands can proceed in several ways. Direct detection can be done in the detection module when the newly synthesized strands comprise ETM labels, either by incorporation into the primers or by incorporation of modified labelled nucleotides into the growing strand. Alternatively, as is more fully outlined below, indirect detection of unlabelled strands (which now serve as “targets” in the detection mode) can occur using a variety of sandwich assay configurations. As will be appreciated by those in the art, it is preferable to detect DNA strands during NASBA since the presence of the ribonuclease makes the RNA strands potentially labile.

[0177] In a preferred embodiment, the amplification technique is signal amplification. Signal amplification involves the use of limited number of target molecules as templates to either generate multiple signaling probes or allow the use of multiple signaling probes. Signal amplification strategies include LCR, CPT, and the use of amplification probes in sandwich assays.

[0178] In a preferred embodiment, the signal amplification technique is LCR. The method can be run in two different ways; in a first embodiment, only one strand of a target sequence is used as a template for ligation; alternatively, both strands may be used. See generally U.S. Pat. Nos. 5,185,243 and 5,573,907; EP 0 320 308 B1; EP 0 336 731 B1; EP 0 439 182 B1; WO 90/01069; WO 89/12696; and WO 89/09835, and U.S. Ser. No. 60/078,102 and 60/073,011, all of which are incorporated by reference.

[0179] In a preferred embodiment, the single-stranded target sequence comprises a first target domain and a second target domain, and a first LCR primer and a second LCR primer nucleic acids are added, that are substantially complementary to its respective target domain and thus will hybridize to the target domains. These target domains may be directly adjacent, i.e. contiguous, or separated by a number of nucleotides. If they are non-contiguous, nucleotides are added along with means to join nucleotides, such as a polymerase, that will add the nucleotides to one of the primers. The two LCR primers are then covalently attached, for example using a ligase enzyme such as is known in the art. This forms a first hybridization complex comprising the ligated probe and the target sequence. This hybridization complex is then denatured (disassociated), and the process is repeated to generate a pool of ligated probes. In addition, it may be desirable to have the detection probes, described below, comprise a mismatch at the probe junction site, such that the detection probe cannot be used as a template for ligation.

[0180] In a preferred embodiment, LCR is done for two strands of a double-stranded target sequence. The target sequence is denatured, and two sets of probes are added: one set as outlined above for one strand of the target, and a

separate set (i.e. third and fourth primer robe nucleic acids) for the other strand of the target. In a preferred embodiment, the first and third probes will hybridize, and the second and fourth probes will hybridize, such that amplification can occur. That is, when the first and second probes have been attached, the ligated probe can now be used as a template, in addition to the second target sequence, for the attachment of the third and fourth probes. Similarly, the ligated third and fourth probes will serve as a template for the attachment of the first and second probes, in addition to the first target strand. In this way, an exponential, rather than just a linear, amplification can occur.

[0181] Again, as outlined above, the detection of the LCR reaction can occur directly, in the case where one or both of the primers comprises at least one ETM, or indirectly, using sandwich assays, through the use of additional probes; that is, the ligated probes can serve as target sequences, and detection may utilize amplification probes, capture probes, capture extender probes, label probes, and label extender probes, etc.

[0182] In a preferred embodiment, the signal amplification technique is CPT. CPT technology is described in a number of patents and patent applications, including U.S. Pat. Nos. 5,011,769, 5,403,711, 5,660,988, and 4,876,187, and PCT published applications WO 95/05480, WO 95/1416, and WO 95/00667, and U.S. Ser. No. 09/014,304, all of which are expressly incorporated by reference in their entirety.

[0183] Generally, CPT may be described as follows. A CPT primer (also sometimes referred to herein as a “scissile primer”), comprises two probe sequences separated by a scissile linkage. The CPT primer is substantially complementary to the target sequence and thus will hybridize to it to form a hybridization complex. The scissile linkage is cleaved, without cleaving the target sequence, resulting in the two probe sequences being separated. The two probe sequences can thus be more easily disassociated from the target, and the reaction can be repeated any number of times. The cleaved primer is then detected as outlined herein.

[0184] By “scissile linkage” herein is meant a linkage within the scissile probe that can be cleaved when the probe is part of a hybridization complex, that is, when a double-stranded complex is formed. It is important that the scissile linkage cleave only the scissile probe and not the sequence to which it is hybridized (i.e. either the target sequence or a probe sequence), such that the target sequence may be reused in the reaction for amplification of the signal. As used herein, the scissile linkage, is any connecting chemical structure which joins two probe sequences and which is capable of being selectively cleaved without cleavage of either the probe sequences or the sequence to which the scissile probe is hybridized. The scissile linkage may be a single bond, or a multiple unit sequence. As will be appreciated by those in the art, a number of possible scissile linkages may be used.

[0185] In a preferred embodiment, the scissile linkage comprises RNA. This system, previously described in as outlined above, is based on the fact that certain double-stranded nucleases, particularly ribonucleases, will nick or excise RNA nucleosides from a RNA:DNA hybridization complex. Of particular use in this embodiment is RNAseH, Exo III, and reverse transcriptase.

[0186] In one embodiment, the entire scissile probe is made of RNA, the nicking is facilitated especially when